

SPINAL CORD REGENERATION IN THE SHARK

by
JOHN BRUCE GELDERD

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DEDICATION

This dissertation is lovingly dedicated to the memory of my father and to my mother, who so often encouraged me to "Persevere with your studies son, you shall never regret it."

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TABLE OF CONTENTS

Acknowledgements.	iii
List of Tables.	v
List of Figures	vi
Abstract.	viii
Introduction.	1
Mammalian Studies	2
Inframammalian Studies.	12
Elasmobranch Studies.	21
Experimental.	26
Results	37
Discussion.	60
Conclusion.	68
Literature Cited.	70
Biographical Sketch	79

LIST OF TABLES

Table

1. Summary of Experimental Paradigm.	28
2. Summary of degenerating nerve fiber counts in descending tracts	45
3. Summary of combined left-right bouton counts on motor horn cell bodies and primary dendrites	47
4. Summary of strength test results following spinal cord transection	53

LIST OF FIGURES

Figure

1. Location of spinal cord sections removed for histological analysis.	30
2. Apparatus for testing strength of axial musculature caudal to the site of spinal cord transection	35
3. Site of spinal cord transection showing cistern lined with ependymal cells at rostral stump of cord, small cisterns within the scar lined with endothelial cells and cisterns in both stumps of cord, some of which enclose severed tip of nerve fibers.	39
A. 10 Days Postoperative	39
B. 30 Days Postoperative	39
C. 90 Days Postoperative	39
4. A. Large severed nerve fibers showing beading near their tips and ending in large spherical globules.	42
B. Nerve fibers in rostral stump of spinal cord beginning their intrusion into the scar at 20 days postoperative	42
C. Nerve fiber tips growing through the scar at 30 days postoperative	42
5. Results of bouton counts as revealed by the Rasmussen stain	49

LIST OF FIGURES (Continued)

Figure

6. A. Polygraph tracing showing undulatory movements caudal to transection site.	55
B. Polygraph tracing showing response of axial musculature following stimulation of barbels of normal animal	55
7. A. Results of strength tests following spinal cord transection	58
B. Results of timed swimming trials following spinal cord transection	58
8. A. Comparison between synapse count on motor horn cell bodies caudal to the lesion and undulatory strength.	67
B. Comparison between synapse count on motor horn cell dendrites caudal to the lesion and undulatory strength.	67

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John Bruce Gelderd

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Chairman: Jerald J. Bernstein, Ph.D.

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The shark presents a unique central nervous system for experimental analysis. The present series of experiments assess the regenerative capacity of the central nervous system in the nurse shark (Ginglymostoma cirratum).

The spinal cord was transected at the mid-thoracic level in 28 nurse sharks. Four animals per group were sacrificed at intervals of 10, 20, 30, 40, 60 and 90 days postoperative. Two groups of fish underwent a subsequent spinal cord retransection at the same site at 90 days postoperative and were sacrificed 10 and 20 days later. Three sections of spinal cord were removed from each shark for histological analysis. A section of spinal cord at the site of lesion was stained using a modified protargol silver stain to assess regeneration across the site of lesion. Another spinal cord section caudal to the lesion site was stained using a

modified Nauta technique to show degenerating descending nerve fibers. The Rasmussen stain was used for the light microscopic demonstration of bouton terminaux on motor horn cells caudal to the lesion. Behaviorally, timed trials for swimming speed and a strength test for axial musculature contraction caudal to the lesion site were performed at five-day postoperative intervals.

Histological analysis showed a neuroglial-pial-ependymal scar joining the stumps of spinal cord. Regeneration across the site of lesion did not occur until 40 to 60 days postoperative. Nerve fibers traversed the lesion site from both stumps of cord and tended to follow blood vessels and glial bridges within the scar. The number of descending long tract nerve fibers reaching an area six spinal segments caudal to the lesion was small (9-13%). Despite this, synaptic terminals on motor horn cells caudal to the lesion showed an increase from 10 to 60 days postoperative (45% of normal at 10 days postoperative to 92% of normal at 60 days postoperative).

Immediately upon recovery from anesthesia, all operated sharks exhibited undulatory movements caudal to the site of transection while at rest which were independent of volitional movements rostral to the lesion. These undulatory

movements increased in strength up to 60 days postoperative at which point they were statistically indistinguishable from the normal strength of axial musculature. Swimming prowess was markedly reduced following spinal cord transection and was never recovered. Undulatory movements were uncontrollable and proved detrimental to swimming ability. The body caudal to the site of lesion remained paralyzed in normal attempts to swim.

Retransection of the spinal cord at 90 days postoperative showed no change in the strength of axial musculature caudal to the lesion, timed trials or the number of boutons on motor horn cells. Comparison between the increase in undulatory strength and increase in synaptic contacts on the motor horn cells caudal to the lesion showed a high correlation ($r=.93$, $P<.01$).

It was concluded that the small amount of regeneration across the site of lesion had no effect on the swimming behavior of the operated sharks. The increase in strength of undulatory movements was attributed to the reestablishment of synaptic contacts on motor horn cells caudal to the lesion by local, segmental sprouting.

INTRODUCTION

Historically, there have been many experiments and theories aimed at the solution to the perplexing problem of central nervous system regeneration. One of the earliest investigators in this area was Ramon y Cajal, who demonstrated that growth of central nervous system fibers in embryos resulted from protoplasmic elongation rather than from fusion of cellular elements (Ramon y Cajal, 1928, 1960). Ramon y Cajal's conclusions raised the question whether similar growth would occur following a transection of the spinal cord of an adult animal. Subsequent studies were undertaken in both mammals and inframammals to assess anatomical and physiological regeneration of the central nervous system.

MAMMALIAN STUDIES

Following the publications of Ramon y Cajal (1928) and until the experiments of Sugar and Gerard (1940), little was added to our knowledge of regeneration in the adult mammalian spinal cord. During this period, numerous reports appeared on the regeneration of fetal rat spinal cords. These experiments met with little or no success and apparent voluntary function was explained as spinal reflex activity. Some abortive regeneration was seen, but the regenerated fibers atrophied before becoming functional (Nicholas and Hooker, 1928). In 1940, Sugar and Gerard succeeded in obtaining structural and functional regeneration in the transected spinal cords of young adult rats. In some of these animals, muscle and nerve transplants had been oriented in a longitudinal plane between the severed cord stumps. These animals were able to sit up, clean their face, responded by squealing when their tail was pinched and exhibited coordinated locomotion. Stimulation of the cerebral peduncles elicited vigorous movements of the hindquarters.

In experiments on spinal cats and dogs (Brown and

McCouch, 1947), the lack of functional regeneration in the spinal cord was attributed to the formation of a dense neuroglial-ependymal scar at the site of lesion. Further study of this scarring phenomenon was done by Windle and his associates (Windle and Chambers, 1950a, 1950b, 1951; Windle, Clemente and Chambers, 1952; Windle, 1955, 1956; Clemente, 1955). After surgical transection of the spinal cord of cats and dogs, a dense neuroglial cap or scar formed over both cut ends of the spinal cord. This scar was composed of fibroblasts and neuroglia. The relatively avascular cicatrix between the two stumps of spinal cord was composed of thick masses of collagenous connective tissue and completely isolated the cut ends of the cord. It was this scar which was thought to prevent regeneration and thus inhibit return of function. Administration of the bacterially derived polysaccharide, Piromen, prevented the development of this dense neuroglial scar and permitted the intrusion of blood vessels into the area of lesion. As a result of this reduction of the scar, nerve fibers were able to cross the loose cellular matrix that formed between the two cut ends of the spinal cord. Freeman (1955) undertook an extensive series of experiments concerning spinal cord regeneration in rats. His experiments lasted for more than 15 years. During this time, spinal cord transection was

accomplished in over 7,000 rats. Anatomic regeneration and normal functional return has occurred in approximately 100 rats. Electrophysiological studies on these rats showed conduction of impulses in both directions through the site of lesion. After complete functional recovery (six months to one year), the "walking paraplegic" rats were injected with procaine directly into the spinal cord at the site of transection. The animal again became completely paraplegic. Retransection of the spinal cord in such rats also returned them to a paraplegic state. Freeman (1955), Littrell et al. (1953) and Littrell (1955) transected the spinal cord in adult cats and contrasted the recovery of non-treated animals to those treated by intravenous injections of Piromen. Cats treated with Piromen exhibited return of function beginning at 2-3 months postoperative and peaked at 9-12 months. After this point, the animals regressed to typical paraplegic behavior by 18 months. Histology showed a dense neuroglial-ependymal scar at the site of transection which was "choking off" the regenerated fiber tracts. There was never any return of sensory function. Non-treated animals did not show any restitution of function or anatomical regeneration. These studies indicated that the neuroglial-ependymal scar prevented regeneration in mammals. However, this same scarring phenomenon also occurred in inframammalian forms that

did indeed show both anatomical regeneration and physiological return of function after spinal cord transection. It was thought, therefore, that perhaps mammalian central nervous system neurons lacked sufficient growth potential to regenerate lost peripheral processes. Levi-Montalcini and Brooks (1960) tested the effects of a protein isolated from the mouse salivary gland upon chick and mouse sensory ganglia in vitro. Within 12 hours after injection of this nerve growth factor (NGF) into the medium, a dense halo of nerve fibers surrounded the explant. Injection of NGF into intact animals produced hyperplasia and hypertrophy of sensory and sympathetic neurons. Scott and Liu (1964) injected NGF and Piromen into kittens after crushing the dorsal columns. There appeared to be a definite, direct correlation between the amount and duration of NGF administration and the regeneration of the sensory fibers. Anatomical regeneration across the site of lesion was confirmed electrophysiologically in these kittens although Scott and Liu did not wait for functional return. Scott et al. (1966) administered NGF to young rats after dorsal root crushing and found an increase of 14% in protein production in dorsal root ganglia as compared to no increase without administration of NGF. Harvey and Srebnik (1967) found anatomical and physiological regeneration with return of function

following spinal cord compression in rats treated with L-thyroxine. Non-treated rats showed no regeneration or return of function. In another rare case of central nervous system regeneration, Adams et al. (1968, 1969, 1971) cut the infundibular stalk in ferrets. Degeneration occurred initially, followed within two weeks by new fibers regenerating from the hypothalamus through the fibrous scar. These fibers carried neurosecretory material. During the one-to three-month postoperative period, fibers grew to a proximal ectopic infundibular process which formed following the lesion. In animals kept alive until 12 months postoperative, the entire neurohypophysis had been reinnervated and was functional.

If a nerve fiber is severed, there appear to be at least three distinct reactions: (1) growth does not occur or it is abortive; (2) the original fiber may regenerate and reform its synaptic contact or (3) adjacent intact nerve fibers or cells may develop collateral sprouts and reinnervate the deafferented tissue (Guth and Windle, 1970). Liu and Chambers (1958) experimentally demonstrated sprouting in the spinal cord of the cat following deafferentation either by adjacent dorsal root section or by corticospinal tract ablation at cranial levels. They observed that paraterminal and collateral sprouting was rather generalized

in areas which had been deafferented and that the amount of this sprouting was determined by the extent of the denervation. Goodman and Horel (1966) showed restricted sprouting of optic tract projections in the rat after occipital cortex removal. Schneider (1970) lesioned the visual cortex or superior colliculus in neonate and adult hamsters. The neonate hamsters showed sprouting in optic tract projections and some sparing in visual discrimination tests in contrast to the adult hamsters which showed little or no anatomical regeneration or return of function. By using histochemical fluorescence techniques, Bjorklund and his colleagues have shown regenerative axon sprouting in the rat mesencephalon following electrolytic lesions (Katzman et al., 1971; Bjorklund and Stenevi, 1971) and in the rat spinal cord following spinal cord compression (Bjorklund et al., 1971).

Raisman's investigations (1966, 1969a, 1969b) in the septal nuclei of the adult rat constitute one of the few ultrastructural studies on collateral sprouting in the mammalian central nervous system. Afferents from two separate pathways converge upon the medial septal nucleus. Fibers originating in the hippocampus pass to the septum through the medial forebrain bundle. Hippocampal fibers terminate exclusively on the dendrites of the septal nuclei

whereas the hypothalamic fibers terminate primarily upon the cell bodies of the septal nuclei. After lesioning the hippocampal input, the remaining afferent septal fiber tract showed a high proportion of axon terminals which made contact with more than their normal share of postsynaptic units. This phenomenon was interpreted as a reinnervation by the remaining hypothalamic input of synaptic sites left open by lesioning of the hippocampal input. This was corroborated by lesioning the medial forebrain bundle which produced degeneration of the remaining synaptic contacts. Raisman then lesioned the hypothalamic input and found sprouting of hippocampal fibers in the septum to fill synaptic sites left by the degenerated hypothalamic input. Moore et al. (1971) has recently duplicated Raisman's study utilizing histochemical fluorescence techniques and has corroborated Raisman's findings. Ultrastructural experiments on rat spinal cord (Bernstein and Bernstein, 1971) have shown similar results. Neurons were deafferented by hemisecting the spinal cord. Shortly after these cells were deafferented, they began to hypertrophy. In particular, the dendrites exhibited profuse branching and irregular swelling. Synaptic spines on the hypertrophied dendrites were also greatly increased in number. Concomitant with this was the establishment of large numbers of axodendritic synapses.

Furthermore, the axons that terminated near the site of lesion appeared to arise by way of axonal sprouting from the region of the spinal cord that was not hemisected. To ascertain if there was any long tract involvement in the regenerative process, a group of rats underwent hemisection of the spinal cord just below T2. Ninety days later, the spinal cord was hemisected on the same side at C5 and the spinal cord at vertebral level T2 underwent histological examination for degeneration. The region immediately rostral to the T2 lesion showed new, degenerating nerve fibers in small amounts. The area was also filled with degenerating axodendritic synapses. These data suggested that the long tracts were indeed partially involved in the regeneration of axons to the neurons proximal to the site of the original lesion. Bernstein stressed, however, that the number of fibers appeared to be low and that the vast majority of new synapses originated from segmental sprouting. This study showed, however, that descending long tract nerve fibers in the spinal cord of the rat are capable of limited regeneration to the area immediately rostral to the lesion site. The regenerating axons in the rat appeared to respond to nonspecific influences of the hypertrophied dendrites and established inappropriate connections. The formation of these synapses then effectively terminated further growth.

of the axons. Bernstein and Bernstein (in press) have also shown limited regeneration of axons rostral to the site of hemisection in the Rhesus monkey spinal cord. Motor horn cell dendrites immediately rostral to the site of hemisection showed varicosities. Regenerating axons made normal as well as aberrant synaptic recombinations with reactive neurons rostral to the lesion. The most frequent type of aberrant synaptic complex was a cup-shaped bouton with a central, large extracellular space between presynaptic and postsynaptic membranes. In another recent ultrastructural study, Lund and Lund (1971) found synaptic adjustment in the superior colliculus following enucleation of neonatal and adult rats. Little change was observed in the number and types of synapses in neonatal rats due to synaptogenesis, but adult rats showed a reinvasion of synaptic sites with an incomplete return to a normal proportion of synaptic types.

Although regeneration in the mammalian central nervous system appears possible, functional return has been shown in only isolated cases. The majority of experiments have shown only abortive regeneration. In the instances of functional return after spinal cord lesion, many are regressive and the animal returns to a paraplegic state. Sprouting then appears to be the rule in the mammalian central nervous system,

with functional regeneration the rare exception. There is, as yet, no clear functional significance to the phenomenon of central nervous system sprouting. McCouch et al. (1955) has implicated sprouting in spasticity following spinal cord transection and Schneider (1970) suggests that sparing of pattern vision behavior in neonatal hamsters is due to sprouting of visual pathways. However, this evidence is only suggestive at best and more definitive studies are required to shed more light on this anatomical phenomenon.

INFRAMAMMALIAN STUDIES

In contrast to the abortive regeneration found in mammals, inframammalian forms have proven to be a fertile area for successful central nervous system regeneration studies. In fact, central nervous system regeneration seems to be the rule in lower forms with abortive regeneration the exception. Central nervous system regeneration in bird embryos has been well documented (Clemente, 1955; Hamburger, 1955) although regeneration in the adult bird occurred only in the visual system (Cattaneo, 1923). Central nervous system regeneration studies in reptiles have centered around the well known phenomenon of tail regeneration in lizards (Clemente, 1964; Hamburger, 1955). The central nervous system of amphibia has shown great regenerative powers. With the noted exception of the adult Anurans, the amphibia have proven comparable to teleosts in central nervous system regeneration. After complete transection of the spinal cord of larval salamanders (Piatt, 1955a, 1955b) or in the axolotl (Kirsche, 1956), extensive regeneration of fiber tracts was observed with a concomitant return of normal

function. In the adult salamander, regeneration was equally vigorous (Piatt, 1955a, 1955b). Regeneration of the spinal cord of the adult newt took place within 30 days with or without injection of Piromen (Drummond, 1954). Regeneration in the frog central nervous system has been restricted to the larval stages (Hooker, 1925). The adult frog has shown abortive regeneration of the spinal cord after transection (Piatt and Piatt, 1958; Clemente, 1964). Regeneration in the frog and toad visual system, however, has been very specific and successful, both anatomically and physiologically (Sperry, 1944; Gaze and Jacobson, 1963; Gaze and Keating, 1969, 1970a, 1970b; Gaze, 1970).

The regenerative capacity of the fish spinal cord ranks high among the vertebrates. This has been shown repeatedly through the almost exclusive use of the teleost as an experimental animal in regeneration studies. Regeneration in cyclostomes has been largely restricted to spinal cord regeneration in larval forms (Maron, 1959; Hibbard, 1963; Niazi, 1963). Spinal cord regeneration in teleosts has proven so superior to the mammalian nervous system that what would be considered poor functional or anatomical recovery in teleosts would undoubtedly be hailed as strikingly successful in man or any commonly used laboratory mammal. Regeneration in teleosts does not occur

to the same degree in all parts of the central nervous system. Regeneration throughout the central nervous system in fish has recently been thoroughly reported by Segaar (1965) and Bernstein (1970) and will not be repeated here. The discussion here will be largely restricted to spinal cord regeneration.

The regenerative capacity of the fish spinal cord extends from the simple regrowth of axons across the site of lesion to the complete restitution of neural cytoarchitectonics, replete with new nerve cells and glia. Koppanyi and Weiss (1922) carried out spinal cord transections at a high level in goldfish. The fish were paralyzed caudal to the lesion for two to three weeks, after which they began to show signs of return of function. After 60 days, the fish were behaviorally indistinguishable from normals. Histological examination showed regeneration of neural pathways which resulted in the reappearance of normal connections (Koppanyi and Weiss, 1922; Koppanyi, 1955). Percy and Koppanyi (1924) later cut the entire vertebral column of goldfish with scissors so that no bony continuity remained between the regions anterior and posterior to the section. Ten weeks postoperatively, the fish were again swimming normally. Hooker (1930, 1932) transected the spinal cord of guppies less than four days old. He claimed full coordination and

integrative movements concomitant with the reestablishment of nervous connections between the two halves of the body approximately four days postoperatively. Keil (1940) transected the spinal cord of adult rainbow fish and claimed restitution of function beginning from three to twelve days postoperative with complete restitution of function at 30-40 days postoperative. Ten years later, Kirsche (1950) confirmed not only the functional but also the morphological regeneration of the spinal cord in the adult rainbow fish. Kirsche introduced the method of stimulating the spinal cord above the site of transection. Tail fin movements were elicited when the cord, and only the cord was stimulated above the transection. Those animals which showed no morphological regeneration also showed no movement of the caudal fin upon stimulation of the spinal cord above the transection. Kirsche distinguished various phases during the course of regeneration. The first phase, which was apparent approximately four days after the transection, consisted of a disorganized growth from the severed stumps. The second phase began about seven days postoperative with a mitotic increase of the ependymal cells to form "indifferent neural cells" which in time developed into neuroblasts and glioblasts. Further differentiation lead to the formation of normal cells in both proximal and distal stumps. Oriented

fibers grew out from these new cells and, approximately 15 days postoperatively, there was evidence for both morphological continuity and functional recovery (Kirsche, 1950, 1965). Healey (1962) transected the spinal cord of minnows and noted the immediate inability of the minnow to change colors upon background color reversal. Fast color changes were shown to be under autonomic control and slow color changes were mediated by hormonal control. Ten days after transection, the fishes ability to change color increased. After four months, rapid color changes occurred that were indistinguishable from normal. Bernstein (1964) has shown a relationship between age and regenerative capacity of the goldfish spinal cord. Young goldfish (less than one year old) were able to reconstitute approximately 90% of the available descending axons whereas approximately 60% were reconstituted in two- and three-year-old animals. The ability of the neuroglia to regenerate and reestablish the diameter of the cord was also age dependent. The younger goldfish reconstituted the diameter of the cord almost completely (Bernstein, 1964). Not only has the spinal cord of the teleost fish regenerated after being severed, it also has the ability to completely reconstitute areas of the spinal cord following ablation. This type of growth pattern has been found in the regeneration of the caudal neurosecretory system of Tilapia. After removal

of the caudal peduncle, tailfin and caudal spinal cord segments, a new caudal neurosecretory system regenerated. This system was somewhat abberant but fully functional (Fridberg et al., 1966).

In a series of experiments on goldfish, it has been shown that although there was return of function following spinal cord transection, the morphological regeneration was less than perfect (Bernstein, 1964; Bernstein and Bernstein, 1968; Bernstein and Gelderd, 1970; Bernstein and Gelderd, in manuscript). Following spinal cord transection, goldfish were paralyzed caudal to the site of lesion, descending spinal tracts degenerated, and synaptic sites on perikaryon and primary dendrites of motor horn cells 2 cm caudal to the site of lesion dropped by 50%. Following 60 days regeneration time, the synaptic complement on motor horn cells was reestablished although descending fiber tract regeneration was only 35-50% of normal. A subsequent retransection of the spinal cord at 60 days postoperative resulted in degeneration of the new, regenerated descending fibers and concomitant paralysis caudal to the site of lesion. In contrast, there was no statistically significant change in the synaptic complement on motor horn cells 2cm caudal to the site of lesion. This seemed to indicate that the descending fibers regenerating into the caudal section of spinal cord did not

return to their original synaptic sites on motor horn cell perikaryon or primary dendrites, but perhaps synapsed instead on internuncial cells. Return of the normal synaptic complement on motor horn cell perikaryon or primary dendrites was relegated to local, segmental sprouting of adjacent fiber tracts or cells. Those descending fibers which did not regenerate past the site of lesion appeared to synapse on cells near the lesion site. When the regenerating axons reached the site of lesion, they were confronted with deafferented neurons. The regenerating axons synapsed on these cells until the synaptic sites were filled. Once the regenerating axons made these inappropriate synaptic contacts, they ceased their growth. The mechanism for the cessation of growth of these axons is thought to be a special case of contact inhibition (Bernstein and Bernstein, 1968). It must be stressed again that regeneration in the goldfish central nervous system is not a uniform phenomenon. Although transection of the spinal cord was followed by a reduced regenerative capacity in the number of fibers traversing the lesion (Bernstein and Gelderd, 1970), lesions of the visual system were followed by a more specific regenerative process which initially appeared to be point for point with 100% of the original optic fibers regenerating (Attardi and Sperry, 1963; Jacobson and Gaze, 1965). Recent studies, however,

have shown some deviation in the area of termination of regenerating optic nerve fibers in the optic tectum of the goldfish following lesions in the visual system (Yoon, 1971; Horder, 1971; Sharma, 1972).

In mammals, the neuroglial-ependymal scar is thought to be responsible in part for the apparent lack of regeneration in the central nervous system, forming a dense barrier between the cut ends of the spinal cord and thus preventing regeneration (Brown and McCouch, 1947; Windle, 1955; Windle and Chambers, 1950a, 1950b; Guth and Windle, 1970). Bernstein and Bernstein (1967) investigated the effect of the neuroglial-ependymal scar on spinal cord regeneration in goldfish. The spinal cord was completely transected and a thin teflon disc was placed between the two cut ends of the spinal cord. The teflon disc remained between the cut ends for 30 days which was ample time for regeneration to occur in goldfish. The goldfish were then operated upon again and the teflon disc removed. These goldfish were observed for an extended period of time to determine any return of function caudal to the lesion which would signify regeneration of the spinal motor tracts. No regeneration or return of function was observed six months later. Other goldfish were operated upon again at 30 days postoperative. The teflon disc was removed and the spinal

cord transected one spinal segment rostral to the original lesion. After 30 days, the spinal cord was observed histologically to assess regeneration. The descending spinal motor tracts grew through the second lesion, caudalward through the isolated section of the spinal cord and through the first lesion which was delineated by a substantial neuroglial-ependymal scar. Hence, the regenerative capacity of the goldfish spinal cord was not affected by the neuroglial-ependymal scar acting as a mechanical barrier (Bernstein and Bernstein, 1967).

ELASMOBRANCH STUDIES

The shark presents a unique nervous system, both among the fishes specifically and vertebrates in general. One trait which is particularly unique to the elasmobranchs is the reported absence of internuncial cells (Golgi Type II) in the spinal cord (Kappers et al., 1936; Von Lenhossek, 1892, 1895; Aronson, 1963; Nieuwenhuys, 1964). Presumably, the vast majority of descending tracts end directly on motor horn cells without intermediary neurons to intercede or modulate information from higher centers. Another unique characteristic restricted to the elasmobranchs is the high urea content in the blood and sera (Goldstein, 1967; Goldstein et al., 1968; Rasmussen and Rasmussen, 1967; Smith, 1929; Rasmussen, 1971). This phenomenon is particularly evident in marine elasmobranchs. This large concentration of urea is also reflected in the cerebrospinal fluid (Smith, 1929; Rasmussen, 1971) and may have a profound effect on the regenerative capacity of the shark central nervous system because urea is used to shrink brain tissue during neurological operations.

In addition to the above-mentioned characteristics of the shark central nervous system, this class of fish shows important advancements in the evolution of the vertebrate central nervous system. The structure of the spinal cord in elasmobranchs may be considered a prototype for that of high vertebrates. Unlike the spinal cord of cyclostomes which is flat, the spinal cord of elasmobranchs is round or oval. In addition, these are the first primitive animals to have myelinated fibers in the spinal cord and whose dorsal and ventral roots unite outside the vertebral column to form a mixed root. It is also in this class that one first finds the division of the gray matter into dorsal and ventral horns and the first time that all cells of origin for sensory fibers in the cord lie in extramedullary spinal ganglia (Kappers, 1906; Kappers et al., 1936; Aronson, 1963; Nieuwenhuys, 1964).

In mammals and other inframammalian forms, transection of the spinal cord produces paralysis immediately after transection. In most inframammalians, paralysis is alleviated by regeneration and return of function after varying periods of time. In the case of mammals, spinal walking may occur after weeks or months of recuperation. The shark, however, has exhibited coordinated undulatory movements immediately upon recovery from anesthesia after

spinal cord transection (Ten Cate and Ten Cate-Kazejawa, 1933; Gray and Sand, 1936a, 1936b; Lissmann, 1946a, 1946b; Healey, 1957) and was able to swim, using coordinated movements between those portions of the body rostral and caudal to the transection. Ten Cate and Ten Cate-Kazejawa (1933) removed all the muscles in the region of the anterior dorsal fin of the dogfish (Scyllium canicula and S. catalus) and transected the spinal cord in the same region. He observed a locomotory rhythm propagated over the site of the operation, thereby maintaining coordinated movement between the head and posterior region of the body. According to Ten Cate and Ten Cate-Kazejawa (1933), the activity of the posterior region of the body depended upon tensile stimuli applied to the posterior musculature whenever an active contraction occurred in the head region. If this conclusion is justified, then the normal locomotory rhythm of the dogfish involves the activity of a chain of peripherally controlled reflexes. Gray (1936) and Gray and Sand (1936a, 1936b) disagreed with the hypothesis of Ten Cate and Ten Cate-Kazejawa. They showed that coordinated responses no longer occurred if two regions of the body of a dogfish were isolated from one another by a second spinal cord transection. If both of these regions were of sufficient length, each exhibited an independent, spontaneous, automatic activity within the

spinal cord. Lissman (1946a, 1946b) showed that locomotory rhythm could only be abolished in spinal dogfish by a complete, bilateral rhizotomy caudal to the transection. This conclusion appeared to support Ten Cate's hypothesis. The continued locomotory rhythm after spinal cord transection in sharks may in part be attributed to its rather low position on the phylogenetic ladder. Eels have shown an undulatory behavior after decapitation, having a duration of only a few seconds (Gray, 1936). Gray has attributed this phenomenon to injury potentials in the remaining spinal cord. Typically, spinal eels laid on their side and showed no coordination between rostral and caudal portions of the body in normal attempts to swim. Nociceptive stimuli did, however, cause undulations (Gray, 1936). Little mention has been made of swimming prowess in the shark with respect to strength and speed after spinal cord transection. An attempt to clarify these questions was made during this series of experiments.

Central nervous system regeneration studies in elasmobranchs are conspicuous by their absence. Although lesion studies have been done (Ten Cate and Ten Cate-Kazejawa, 1933; Gray and Sand, 1936a, 1936b; Lissman, 1946a, 1946b; Healey, 1957; Segaar, 1965; Ebbesson, 1972), there have been no known experiments concerning

shark central nervous system regeneration. Indeed, experimental work per se on the shark central nervous system is at best limited. It is this complete lack of central nervous system regeneration experiments on sharks plus the anatomical and behavioral uniqueness of their central nervous system that have prompted me to use them as experimental animals. It was my intent to use these unique features of the shark central nervous system to shed some light on the regenerative process.

EXPERIMENTAL

Materials and Methods

Subjects

Thirty-six male and female nurse sharks (Ginglymostoma cirratum), approximately two feet in length, were used. These sharks were trapped in the coastal waters off Ft. Lauderdale, Florida.

Environment

All fish were kept at Marineland of Florida in an outside, circular, salt water tank 15 feet in diameter and six feet in depth. A constantly circulating salt water system was used to insure proper oxygenation, salinity and water temperature. Sharks were fed to satiation daily on cut-up fish.

Operative Procedures

All operated fish were anesthetized with Tricaine methanesulfonate (MS-222, 1:4000, Finquel, Ayerst Laboratories), then placed on an operating board. A longitudinal incision was made at the midline in the midthoracic region at the level of the trailing edge of the pectoral fin, and the musculature

dissected away to expose the spinal column. A laminectomy was performed and the spinal cord transected with a scalpel. The wound was sutured and powdered sulfathiazole-sulfonilimide was applied to the suture line to prevent infection. Following surgery, all animals were also given a 0.1 cc intramuscular injection of Longicil. Animals were tagged for identification by attaching a numbered clamp and colored streamers to the anterior dorsal fin. All sharks, including four normals, were separated into nine groups (four animals per group) and were killed by anesthetizing them at 10, 20, 30, 40, 60 and 90 days postoperative and perfusing them with 10% buffered formalin. Two groups of animals underwent a subsequent retransection of the spinal cord at the same site at 90 days postoperative and one group each was killed by the above fixation method at 10 and 20 days postoperative (Table 1).

Histology

Three sections of the spinal cord were removed from each shark: a 2 cm section at the site of lesion, a second section (1 cm in length) six spinal segments caudal to the site of lesion and a 1 cm section immediately caudal to the second section (Fig. 1).

The 2 cm section of spinal cord at the site of lesion was sectioned horizontally at 15 μ and stained using a modified Bodian silver technique counterstained with

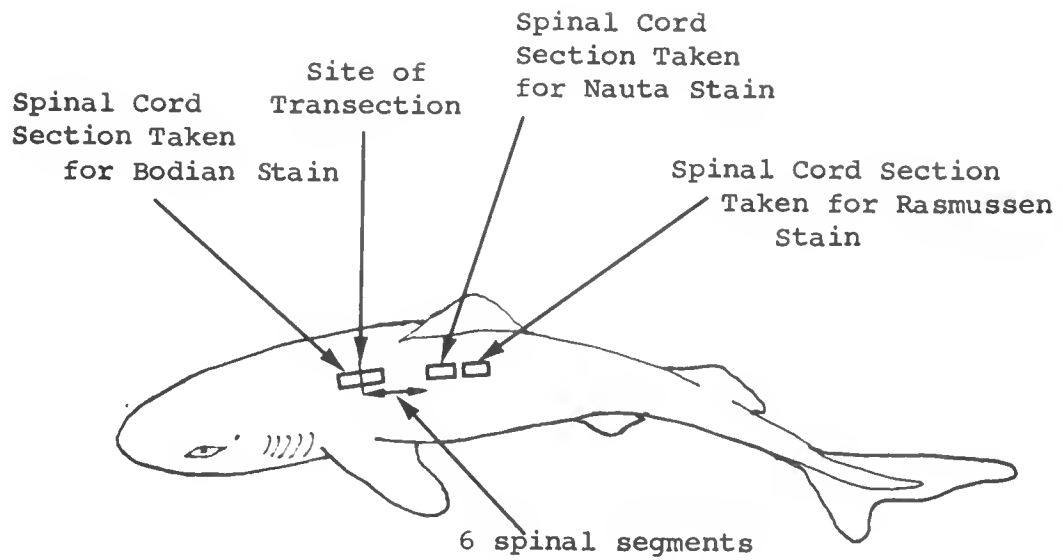
TABLE 1

SUMMARY OF EXPERIMENTAL PARADIGM

Group Number	Number of Animals	Spinal Cord Transection	Sacrifice Day Following Transection	Bodian Stain (Nerve Fiber)	Nauta Stain (Degen. Fiber)	Rasmussen Stain (Synapses)	Behav. Tests Five Day Intervals
1 Normal	4	No	N/A	X Normal		X Normal	X Normal
2	4	Yes	10	X	X	X	X
3	4	Yes	20	X	X	X	X
4	4	Yes	30	X		X	X
5	4	Yes	40	X		X	X
6	4	Yes	60	X		X	X
7	4	Yes	90	X		X	X
8	4	Yes	10*	X	X	X	X
9	4	Yes	20*	X	X	X	X
TOTAL		36					

NOTE: These fish underwent a subsequent spinal cord retransection at the same site at 90 days postoperative. This number indicates the sacrifice day following the second transection.

Figure 1 - Location of spinal cord sections removed for histological analysis.



cresyl-violet and eosin. The regenerative process was assessed with respect to (1) formation of a neuroglial-ependymal scar, (2) the effect of the scar on regeneration and (3) the rate of the regenerative process.

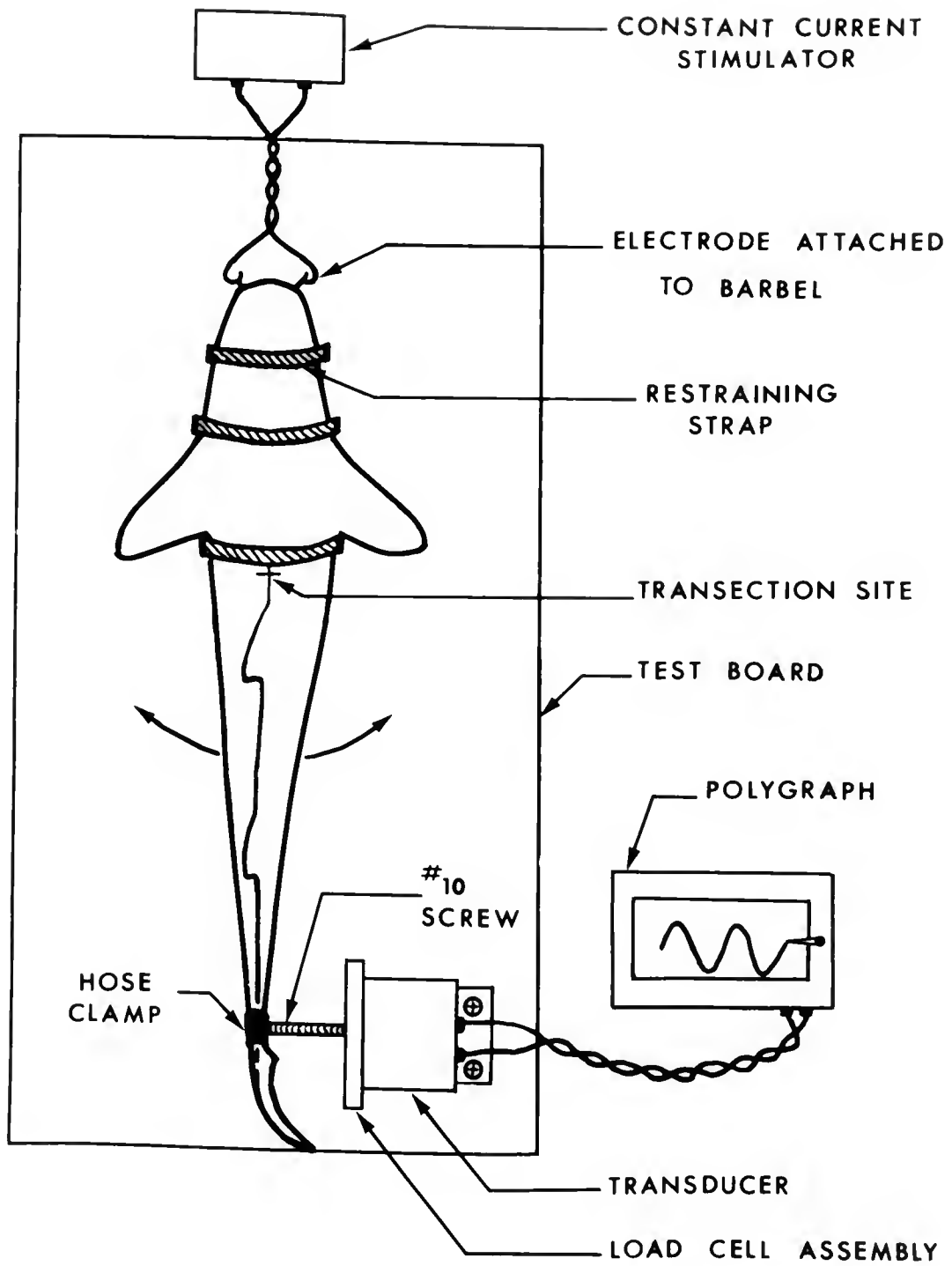
Three descending tracts (tectospinal, thalamospinal and ventral cerebellospinal tract) were studied in the 1 cm segment of spinal cord located six spinal segments caudal to the site of lesion. This section of spinal cord was histologically analyzed with respect to the number of degenerating nerve fibers within a given tract following spinal cord transection (Fig. 1). The spinal cord was serially sectioned horizontally at 30 μ and impregnated using a modified Nauta technique for degenerating nerve fibers. The degenerative pattern was plotted by drawing a composite cross-sectional diagram made by examining, in order, each horizontal section. The histological sections corresponding to the known anatomical locations of the given descending tracts were selected and the number of degenerating fibers counted in each tract at 10 and 20 days postoperative. These data were compared to the number of degenerating nerve fibers found 10 and 20 days following the subsequent retransection (Table 1). The second transection controlled for local versus long tract input following regeneration.

A quantitative analysis of the synaptic terminals was done on the perikaryon and primary dendrites of ventral motor horn cells following spinal cord transection. The section (1 cm in length) of spinal cord used was taken immediately caudal to the section used for fiber tract analysis so that the number of regenerating fibers and the synaptic profiles could be compared (Fig. 1). The spinal cord was sectioned coronally at 10 μ and impregnated using the Rasmussen stain for the light microscopic demonstration of bouton terminaux (Rasmussen, 1957) and followed by a cresyl-violet and eosin counterstain. Counts were made on only those motor horn cells in which a prominent nucleolus and primary dendrite could be seen in a given section. Counts were made on a total of 576 motor horn cells. Sixteen motor horn cells were counted per shark, utilizing eight cells on the left side and eight cells on the right side. Synaptic terminals were counted at 10, 20, 30, 40, 60, and 90 days postoperative and on the two groups of fish retransected at 90 days postoperative (Table 1). All counts were made on coded slides to insure unbiased results. The resultant data were decoded and the levels of significance determined for intra- and intergroup interactions by using computer program BMDX63 for multivariate analysis of variance.

Behavior

A behavioral analysis of the sharks was done during the postoperative period including the two groups retransected at 90 days. The operated sharks were observed daily while swimming in the tank and compared to normal sharks with respect to swimming prowess. In addition, two quantitative tests were performed on all sharks, preoperatively and at five-day postoperative intervals. The first test consisted of removing each shark from the tank and strapping it to a board with that portion of the body rostral to the lesion firmly held in place. That portion of the body caudal to the lesion remained unrestrained with the exception of the caudal peduncle to which a hose clamp was attached. The clamp was connected by way of a #10 screw to a Statham load cell assembly (Model UL-4) which was in turn mounted on a Statham universal force transducer (Model UC-3). The entire transducer assembly was securely mounted on the test board. The output of the force transducer was fed into a two-channel Grass polygraph recorder. Two electrodes were attached to the paired barbels located on the underside of the snout of the shark (Fig. 2). The shark was stimulated using a constant current stimulator producing a 10 ma pulse of 50 msec duration. The strength of the response of the caudal body musculature

Figure 2 - Apparatus for testing strength of axial musculature caudal to the site of spinal cord transection.



following stimulation was recorded on the polygraph and compared to preoperative and normal data. A minimum of five responses was recorded for each shark on a given trial day.

The second test consisted of timed swimming trials. Each shark was placed in the water at one end of a 7' x 3' x 2' tank and the time required for the shark to swim the length of the tank was recorded. Two consecutive timed trials were measured on each shark on a given trial day. Only those trials were counted in which swimming was uninterrupted over the entire distance. The postoperative data for both behavioral tests were compared to normal data in addition to intra- and intergroup interactions among postoperative groups by utilizing computer program BMD08V for analysis of variance.

RESULTS

Histological

Site of Lesion

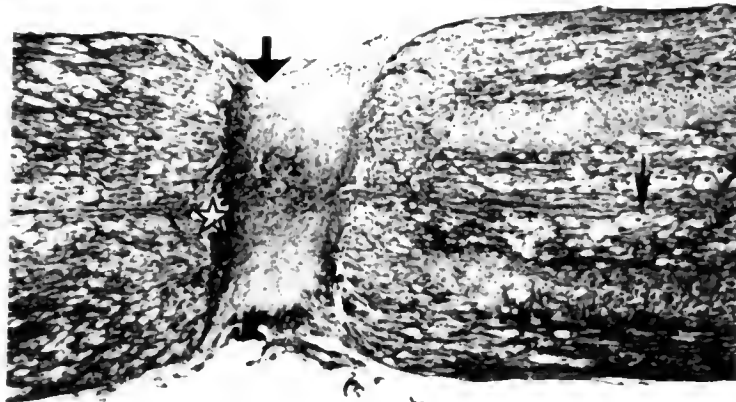
Histological analysis at the site of lesion in the group of animals sacrificed at 10 days postoperative showed a dense scar separating the cut ends of the spinal cord (Fig. 3A). The diameter of the cord in the scar area was approximately 75% of normal. The scar was composed of neuroglial, ependymal and pial cells. Blood cells and phagocytes were also present in abundance throughout the scar, but no blood vessels were seen within the scar at this time. Three types of cisterns were found within or near the site of lesion. A cistern lined with ependymal cells was visible at the rostral stump of spinal cord. Several smaller cisterns lined with endothelial cells were also present throughout the scar. A third type of space or cistern was found near the lesion site in both stumps of spinal cord. These spaces were not lined with cells and some had severed nerve fiber tips within them. All three types of cisterns were seen in the lesion

Figure 3 - Site of spinal cord transection showing cistern lined with ependymal cells (☆), at rostral stump of cord, small cisterns within the scar (▼) lined with endothelial cells and cisterns in both stumps of spinal cord (▼) some of which enclose severed tips of nerve fibers. Bodian silver stain (X10).

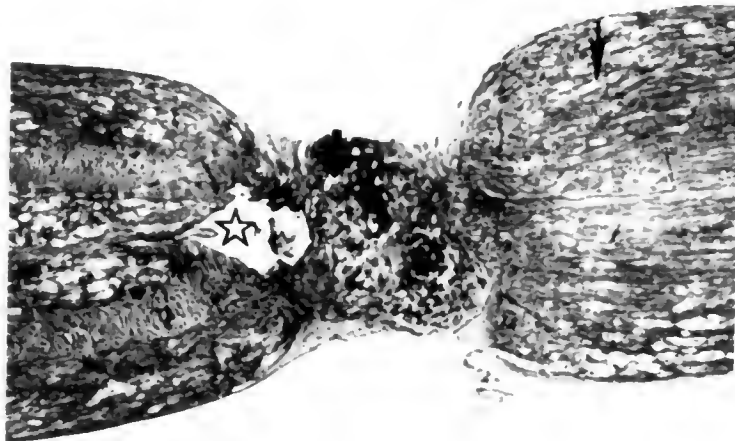
A. 10 days postoperative

B. 30 days postoperative

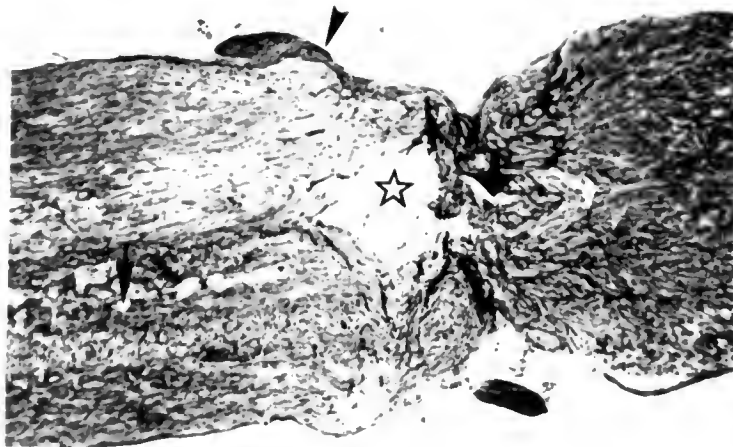
C. 90 days postoperative - cut ventral root growing from rostral stump of cord into scar area (▼).



A



B



C

area during the entire postoperative period (Fig. 3A, 3B, 3C). No nerve fibers were found within the scar at this time. Many large nerve fiber tips were, in fact, found well back from both cut ends of spinal cord (Fig. 3A). These large nerve fibers were beaded at their terminals and ended in large spherical globules (Fig. 4A). This phenomenon persisted throughout the postoperative period.

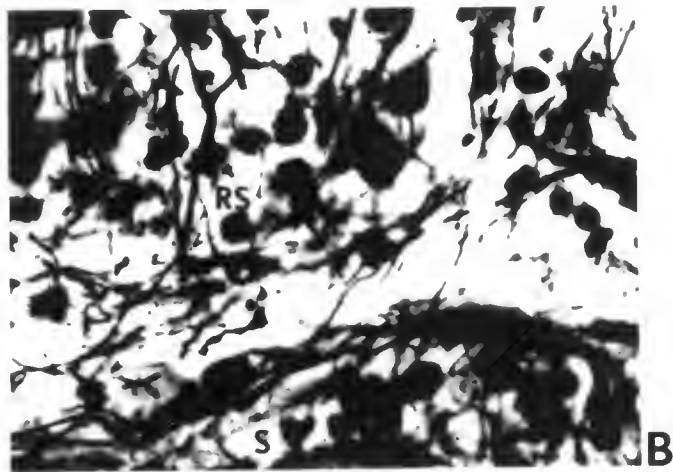
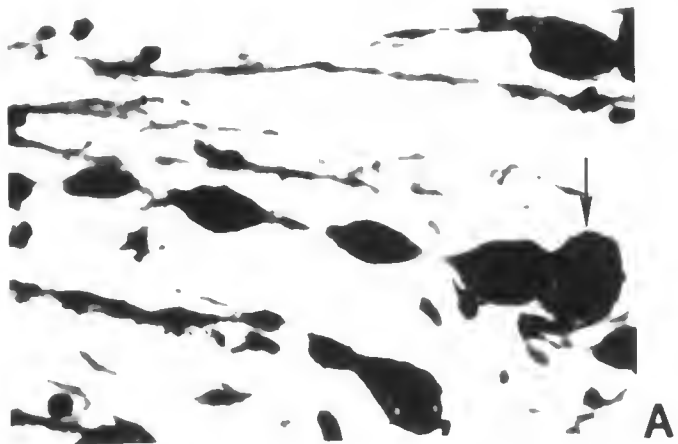
At 20 days postoperative, the scar area resembled that at ten days. Nerve fibers were seen immediately adjacent to the scar with some fibers beginning their intrusion into the scar from both stumps of cord (Fig. 4B).

At 30 days postoperative the cistern lined with ependymal cells had increased considerably in size and the diameter of the scar was further reduced (55% of normal). Nerve fibers were seen penetrating the scar from both stumps of cord (Fig. 4C) but the center of the scar was still devoid of nerve fibers.

At 40 days postoperative there was little change in the appearance of the scar or the density and intrusion of nerve fibers into the scar area.

There were no discernable differences in the appearance of the lesion site between 60 and 90 days postoperative. The scar appeared as a loose cellular matrix made up of neuroglial and ependymal cells. The ependyma-

- Figure 4 - A. Large severed nerve fibers showing beading near their tips and ending in large spherical globules (↓). Bodian silver stain (X1,000).
- B. Nerve fibers in rostral stump (RS) of spinal cord beginning their intrusion into the scar (S) at 20 days postoperative. Bodian silver stain (X450).
- C. Nerve fiber tips growing through the scar at 30 days postoperative. Bodian silver stain (X1,000).



lined cistern at the rostral stump of cord had increased still more in size (Fig. 3C) but no further constriction in the diameter of the scar was observed. The increase in the size of the ependyma-lined cistern in the rostral stump of spinal cord was probably due to blockage of the flow of cerebrospinal fluid in the central canal of the spinal cord. There was a large increase in the number of nerve fibers within the site of lesion. These nerve fibers were of small caliber and appeared to completely traverse the scar by following neuroglial bridges and blood vessels. In those sharks where the transection was made immediately caudal to the emergence of a pair of ventral roots, the ventral roots were severed in the process of transecting the cord. Severed ventral roots were seen, at 60 and 90 days postoperative, growing caudally along the edge of the cord until they reached the lesion site where they grew into the scar and the caudal stump of spinal cord (Fig. 3C). Nerve cell bodies were not found within the site of lesion at any time during the postoperative period.

Nauta Stain

In the process of counting the number of fibers in the three descending tracts following spinal cord transection, it was determined that the tectospinal and thalamospinal tracts could not be effectively counted

separately because they were anatomically adjacent to one another within the spinal cord. Consequently, they were counted as one tract. There was little evidence of degeneration 10 days postoperative to both the first transection and the subsequent retransection. This agreed with lesion studies in the shark visual system utilizing the Nauta technique where signs of degeneration following lesions did not occur until approximately 20 days postoperative (Ebbesson and Ramsey, 1968; Ebbesson and Schroeder, 1971). Those sharks sacrificed at 20 days following both transections showed degeneration within the three descending tracts and were thus used to make the counts.

Degenerating descending nerve fibers showed typical irregular beading and droplet formations with concomitant phagocytic activity typical of lower vertebrates. Ascending tracts showed no retrograde degeneration.

Nerve fiber counts in the ventral cerebellospinal tract and the combined tectospinal-thalamospinal tracts are summarized in Table 2. Degenerating nerve fibers counted 20 days following the first transection represent the normal complement of axons in each of the respective tracts. Those nerve fibers counted 20 days following the retransection at 90 days postoperative were the number of regenerated axons that originated rostral to the lesion site. The number of

TABLE 2
SUMMARY OF DEGENERATING NERVE FIBER
COUNTS IN DESCENDING TRACTS

Tract	Numbers of Fibers		
	Normal	Regenerated 90 Days	% Regenerated 90 Days
Ventral Cerebellospinal	774	72	9.3
Tectospinal-Thalamospinal	1584	213	13.4

degenerating nerve fibers found within the combined tectospinal-thalamospinal and ventral spinocerebellar tracts following the second transection were 13.4% and 9.3% respectively of the number of degenerating nerve fibers found following the first transection.

Rasmussen Stain

The results of the synaptic terminal counts as revealed by the Rasmussen stain on cell bodies and primary dendrites of motor horn cells caudal to the lesion are summarized in Table 3 and Fig. 5. The average number of boutons represented was the combined data on both the left and right sides of the spinal cord since there were no statistically significant differences ($P > .05$) between right and left counts. In addition, there was a high correlation ($r = .974$, $P < .01$) between bouton counts on cell bodies and primary dendrites.

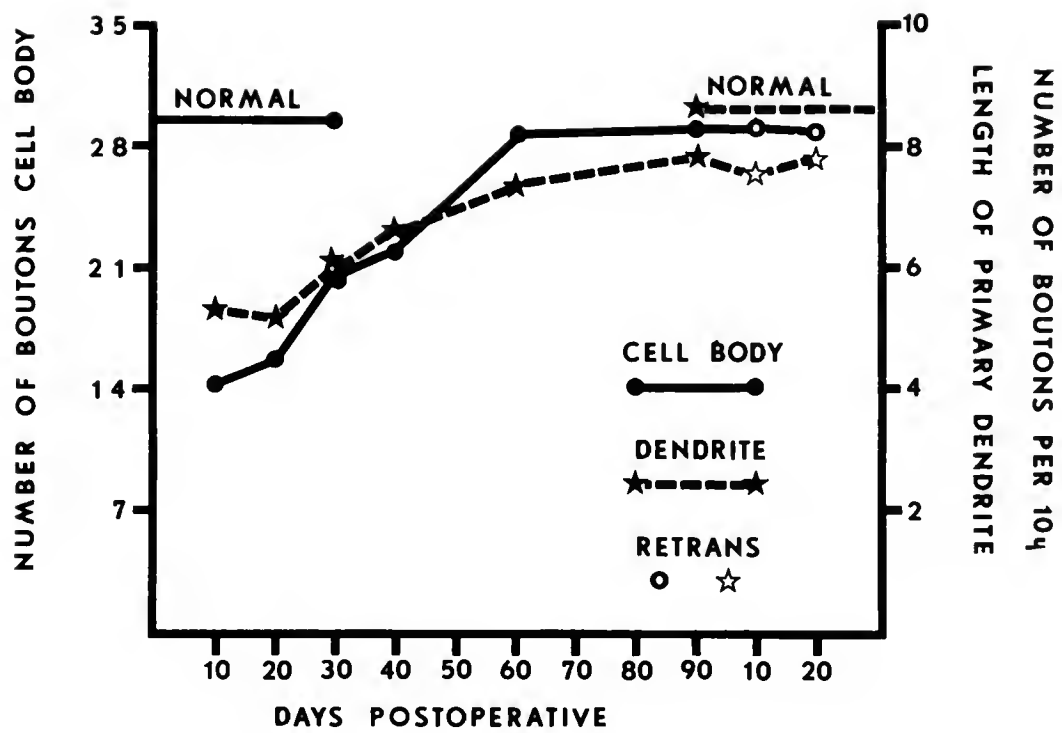
The number of boutons on cell bodies were statistically less than normal ($P < .05$) throughout the postoperative period, including the retransected groups. There was, however, a statistically significant increase in boutons with time after surgery from 10 to 60 days ($P < .05$), but no statistically significant difference occurred between 60, 90 and the two retransected groups ($P > .05$). Synaptic terminals dropped to 45% of normal at 10 days

TABLE 3

SUMMARY OF COMBINED LEFT-RIGHT BOUTON COUNTS
ON MOTOR HORN CELL BODIES
AND PRIMARY DENDRITES

Days Postoperative	Combined Left-Right Cell Body Count (\bar{X} ±SEM)	% of Normal	Combined Left-Right Count Per 10µ Primary Dendrite (\bar{X} ±SEM)	% of Normal
10	14.15±0.82	45.06	5.30±0.49	62.27
20	17.50±0.47	54.61	5.09±0.98	59.81
30	20.50±0.72	65.28	5.92±0.42	69.56
40	23.86±0.25	75.98	6.58±0.17	77.32
60	28.96±0.32	92.22	7.30±0.38	85.78
90	29.90±0.12	95.22	7.78±0.37	91.42
RETRANSECTION AT 90 DAYS POSTOPERATIVE				
10	29.90±0.49	95.22	7.42±0.39	87.19
20	29.78±0.49	94.84	7.80±0.27	91.65
Normal	31.40±0.48		8.51±0.15	

Figure 5 - Results of bouton counts as revealed by the Rasmussen stain. There was a high correlation ($r=.97$, $\underline{p} < .01$) between counts on motor horn cell bodies and primary dendrites.



postoperative, increased to 92% of normal by 60 days postoperative and were 95.2% of normal at 90 days postoperative. The retransected groups sacrificed at 10 and 20 days following retranssection were 95.2% and 94.8% of normal respectively..

The number of boutons per 10 μ primary dendrite following spinal cord transection were significantly less than normal from 10 to 60 days postoperative (\underline{P} <.05) and were significantly greater (\underline{P} <.05) with increased time (62.2% of normal at 10 days, 85.7% of normal at 60 days). The bouton count was 91.4% of normal and statistically indistinguishable from normal (\underline{P} >.05) at 90 days postoperative. Ten days following the retranssection, however, the number of boutons was 87.2% of normal and statistically less than normal (\underline{P} <.05). In contrast, there was no statistically significant difference between normals and 20 days following the retranssection (\underline{P} >.05).

BEHAVIOR

Daily Observations

Immediately upon recovery from anesthesia, all operated sharks exhibited undulatory movements caudal to the site of spinal cord transection. This phenomenon persisted throughout the postoperative period and was never observed in normal animals. Although these undulatory movements occurred caudal to the lesion, there were no swimming movements caudal to the lesion when the animals attempted to swim. Forward movement was accomplished by "walking" along the bottom of the tank using the pectoral fins or by jerking the body rostral to the lesion left and right while dragging the caudal portion of the body. Turning could only be done by walking movements of the pectoral fins.

The undulatory movements during the early postoperative days (1-30 days) were not strong enough to move the operated animals. From 30 days postoperative, however, undulatory movements became strong enough to propel the sharks forward. The animals did not appear able to control these undulatory movements. As a result,

the undulatory movements proved detrimental to their swimming ability. Stimulation caudal to the lesion site by gentle prodding or by an inadvertant touch by another shark caused an increase in undulatory strength which either flipped the shark over on its back, using the snout as a pivotal point, or pushed the startled animal into a wall despite its best efforts to prevent this by using the pectoral fins to "backpedal" away from the wall. The sharks remained paralyzed caudal to the transection for the duration of the postoperative period with respect to normal attempts to swim.

Quantitative Tests

The results of the strength tests and timed swimming trials are summarized in Table 4.

The strength tests (Fig. 2) showed two types of responses. The first response was elicited following stimulation and was in the form of a single, sharp flexure of the axial musculature. A second, consecutive stimulation elicited the same type of response in the opposite direction (Fig. 6B). This response following stimulation was present in both normal and operated animals, although much reduced in the operated animals. This response in operated animals was probably due to passive conduction of movements caused by muscle contractions rostral to the lesion. The second type of response was the previously mentioned undulatory

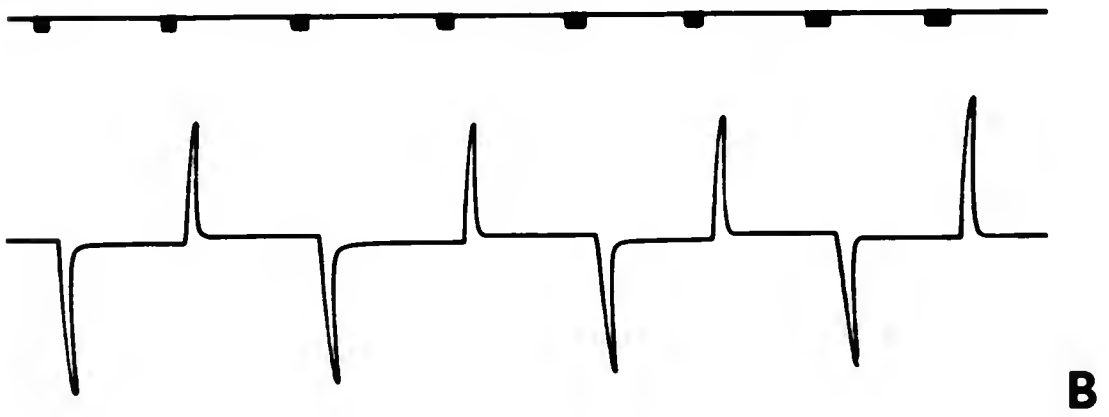
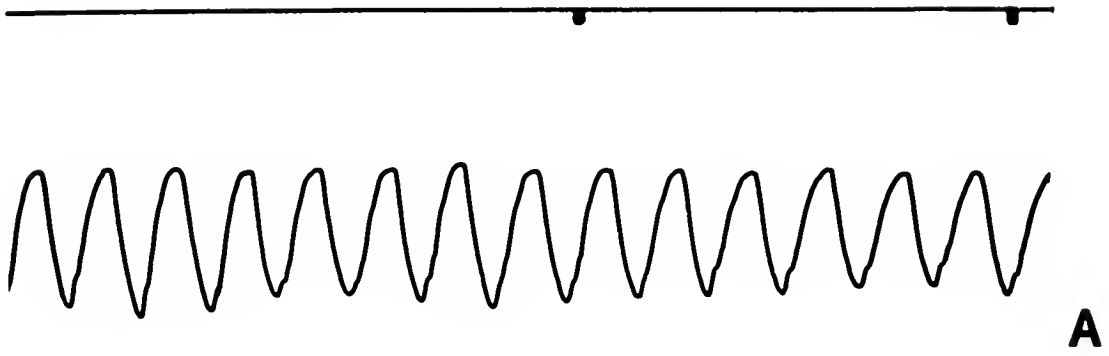
TABLE 4

SUMMARY OF STRENGTH TEST RESULTS
FOLLOWING SPINAL CORD TRANSECTION

DAYS POSTOPERATIVE	UNDULATORY STRENGTH IN KGMS ($\bar{X} \pm \text{SEM}$)	STRENGTH OF RESPONSE FOLLOWING STIMULATION IN KGMS ($\bar{X} \pm \text{SEM}$)	% OF NORMAL	TIMED SWIMMING TRIALS IN SECONDS ($\bar{X} \pm \text{SEM}$)	% OF NORMAL
1	0.08±0.01	0.32±0.04	10.63	9.30±0.30	16.12
10	0.21±0.03	0.29±0.03	9.63	8.86±0.27	16.70
15	0.21±0.02	0.32±0.04	10.63	8.73±0.44	16.95
20	0.26±0.02	0.27±0.03	8.97	8.89±0.38	16.64
25	0.37±0.04	0.34±0.04	11.29	9.84±0.47	15.04
30	0.47±0.06	0.31±0.02	10.29	8.70±0.44	17.01
35	0.87±0.12	0.35±0.03	11.62	8.06±0.37	18.36
40	1.14±0.12	0.38±0.04	12.63	7.85±0.30	18.85
50	1.77±0.18	0.48±0.05	15.94	7.84±0.28	18.87
55	1.96±0.24	0.50±0.05	16.61	8.43±0.36	17.55
60	2.66±0.30	0.49±0.03	16.27	8.44±0.20	17.53
65	2.13±0.23	0.34±0.02	11.29	8.73±0.31	16.95
70	2.23±0.21	0.35±0.02	11.62	8.17±0.38	18.11
75	2.62±0.63	0.36±0.05	11.96	9.17±0.55	16.13
80	2.67±0.20	0.35±0.03	11.62	8.71±0.32	16.99
85	2.60±0.18	0.36±0.01	11.96	9.08±0.19	16.29
90	2.46±0.18	0.38±0.02	12.63	9.03±0.09	16.38
RETRANSECTION AT 90 DAYS POSTOPERATIVE					
1	2.28±0.16	0.31±0.01	10.29	10.45±0.17	14.16
10	2.32±0.19	0.31±0.02	10.29	9.75±0.32	15.17
20	2.44±0.18	0.32±0.01	10.63	9.66±0.26	15.32
Normal	N/A	3.01±0.32		1.48 0.22	

Figure 6 - A. Polygraph tracing showing undulatory movements (0.25-0.5 cycles/sec) caudal to transection site. The top trace indicates when stimulation occurred. Paper speed = 3mm/sec.

B. Polygraph tracing showing response of axial musculature following stimulation of the barbels of a normal animal. The top trace indicates when stimulation occurred. Paper speed = 3mm/se



movements caudal to the site of lesion. These undulatory movements occurred only in operated animals and were an almost constant phenomenon requiring no stimulation (Fig. 6A).

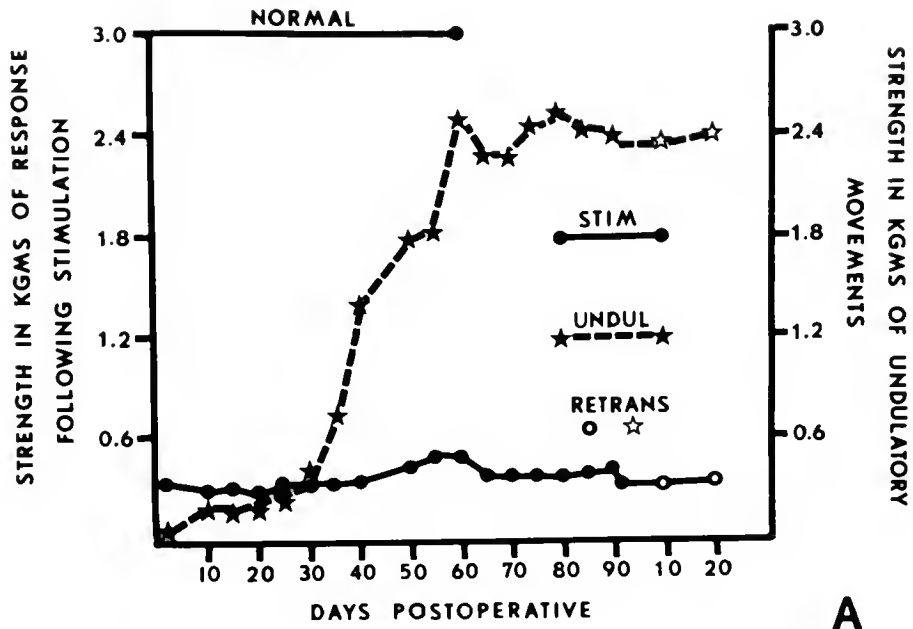
The response following stimulation was significantly reduced from normal throughout the postoperative period ($P < .001$) following the first transection and there was no trend during this postoperative period for any return of strength caudal to the lesion. There was also no statistically significant difference ($P > .05$) between the response following stimulation at 90 days postoperative and the retransected groups (Fig. 7A).

Undulatory movements (0.25-0.5 cycles/sec) were weak during the early postoperative period, but there was a statistically significant increase in the strength of undulatory movements with increased time from 20 to 60 days postoperative ($P < .05$). From 60 days postoperative to 20 days following the retransection, there were no significant differences between postoperative groups ($P > .05$). In addition, the strength attained by undulatory movements at 60 days postoperative was statistically indistinguishable ($P > .05$) from the strength of response following stimulation of normal animals (Fig. 7A).

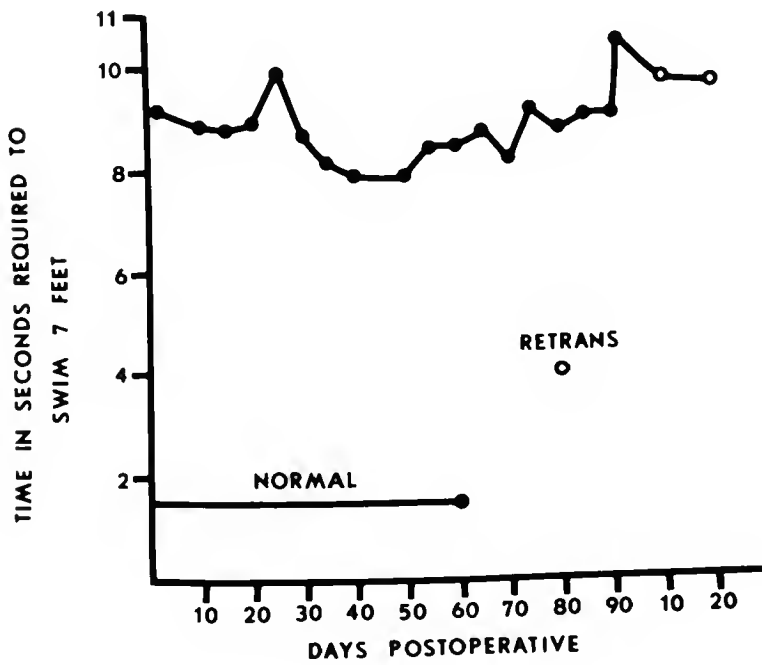
A significant increase was observed in the time

Figure 7 - A. Results of strength tests following spinal cord transection.

B. Results of timed swimming trials following spinal cord transection.



A



B

required to swim seven feet following spinal cord transection ($\underline{P} < .001$). Normal animals required an average of 1.48 seconds, whereas the mean value for transected animals was 8.72 seconds. There was no trend during the postoperative period for improvement in swimming times (Fig. 7B) and there were no significant differences between times following the first and second spinal cord transections ($\underline{P} \geq .05$).

DISCUSSION

These results indicate that following spinal cord transection, the nurse shark is capable of limited anatomical regeneration of descending tracts across the site of lesion (9.3-13.4%) to an area six spinal segments caudal to the lesion. The time required for nerve fibers to regenerate across the lesion site was between 40 and 60 days. This was considerably slower and more incomplete than regeneration in the teleost spinal cord. Teleosts have shown anatomical regeneration and return of normal swimming function from four days postoperative in guppies (Hooker, 1930, 1932) to approximately 35 days postoperative in goldfish (Bernstein, 1964) although descending fiber tracts in the goldfish regenerate only 35-49% of the original complement of fibers within a given tract (Bernstein and Gelderd, 1970).

Limited regeneration did occur in the shark but there was no return of strength in the axial musculature caudal to the spinal cord transection following stimulation rostral to the lesion. The operated sharks were paralyzed caudal to the lesion in normal attempts to swim. This lack of

strength caudal to the lesion was also reflected in the timed swimming trials as there was no trend during the postoperative period for improvement in swimming times. In fact, the small amount of anatomical regeneration seen six spinal segments caudal to the lesion at 90 days postoperative had little or no effect on strength or swimming speed as a subsequent retransection at 90 days postoperative caused no change in these performance parameters in the ensuing postoperative period.

As was stated previously, only a small number of nerve fibers regenerated rostro-caudal across the site of lesion to the area six spinal segments caudal to the lesion and they did not traverse the lesion site until 40 to 60 days following transection. Despite this, the number of synapses on motor horn cell bodies and primary dendrites caudal to the lesion showed an increase beginning at 20 days postoperative through 60 days postoperative and were highly correlated ($r=.974$, $p<.01$).

If synaptic return occurs before the return of long tract input, what is the source of the increase in the number of synapses on motor horn cells caudal to the lesion? The high correlation between primary dendrite and cell body synaptic counts strongly indicates that both phenomena have the same origin. The origin for the nerve fibers which

replaced lost synaptic contacts must have been caudal to the site of lesion in the form of local, segmental sprouting. This hypothesis is further supported by data following the retransection at 90 days postoperative. There was no significant change in the number of boutons on motor horn cell bodies following retransection and a small but statistically significant ($P < .05$) drop (91.4% to 87.6% of normal) in boutons on motor horn cell primary dendrites. This slight drop in boutons on primary dendrites following retransection was probably due to degeneration of the small number of regenerated long tract nerve fibers which synapsed on motor horn cell dendrites.

If there is no return of swimming prowess and no return of axial musculature strength following stimulation, then the question arises as to the functional significance of this sprouting phenomenon and the return of synaptic contacts on motor horn cells caudal to the lesion site. Perhaps the answer to this question lies in the unique appearance in sharks of undulatory movements caudal to the lesion following spinal cord transection. Before this relationship is discussed, however, the anatomical basis for the undulatory movements will be elaborated.

Unlike other shark studies which claim coordinated undulatory movements propagated rostro-caudally over the

site of lesion (Ten Cate and Ten Cate-Kazejawa, 1933; Gray, 1936; Gray and Sand, 1936a, 1936b), undulatory movements in this experiment were observed only caudal to the lesion site and were independent of body movements rostral to the lesion. In fact, during the strength tests, the response following stimulation was often superimposed upon undulatory movements without affecting the speed or strength of the undulatory movements.

There are at least two hypotheses relating to the anatomical basis of undulatory movements in spinal sharks. Ten Cate and Ten Cate-Kazejawa (1933) claim that the undulatory movements are propagated over the lesion site by tensile stimuli applied to posterior musculature when an active contraction occurs in the head region, implying the activity of a chain of peripherally controlled reflexes. Gray (1936) and Gray and Sand (1936a, 1936b) showed that coordinated responses did not occur if two regions of the body of a dogfish were isolated from one another by a second spinal cord transection. Each isolated section of the body exhibited a spontaneous, independent undulatory activity. Gray and Sand attributed this to an inherent undulatory discharge rhythm within the spinal cord. Lissman (1946a, 1946b) has shown that undulatory movements caudal to a spinal cord transection in dogfish can only be

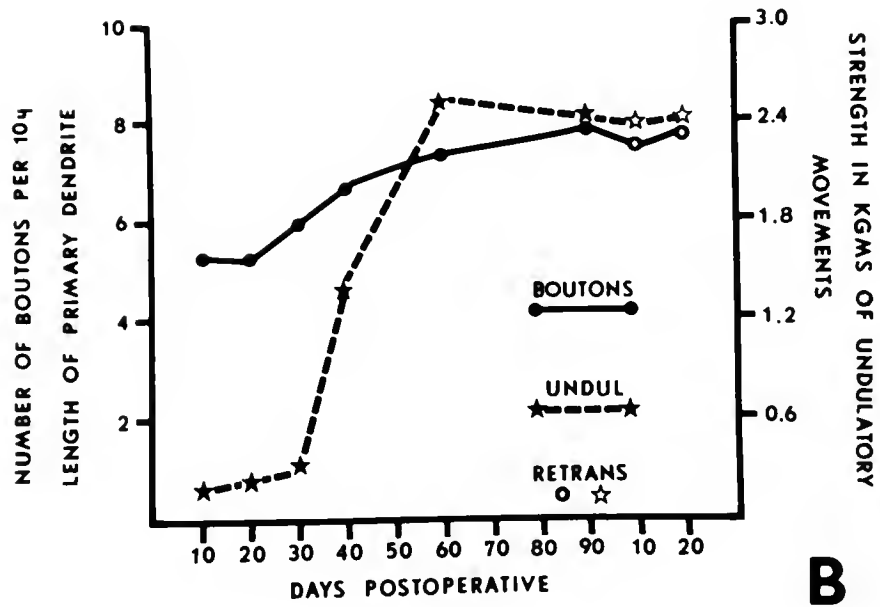
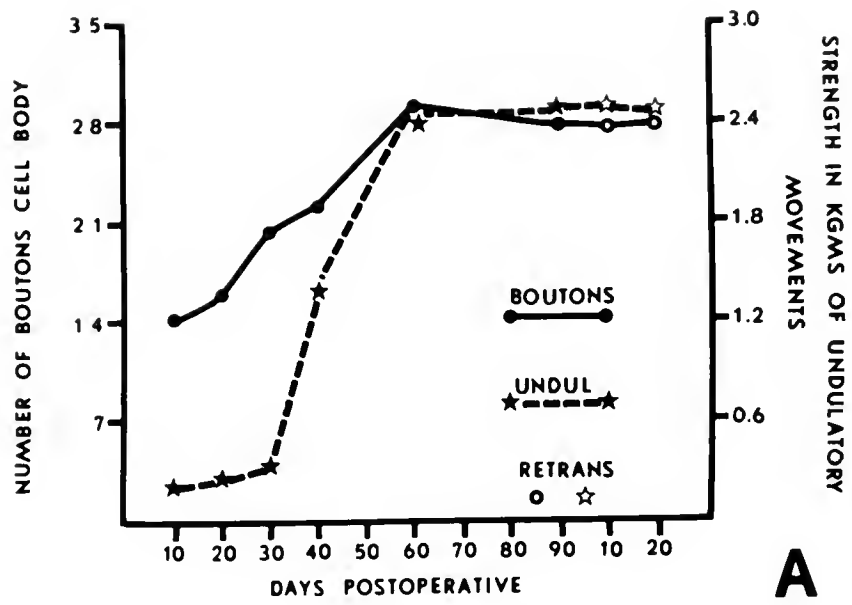
abolished by a complete bilateral rhizotomy caudal to the lesion. The hypothesis of Ten Cate and Ten Cate-Kazejawa (1933) must be rejected in the present experiment because undulatory movements persisted without movement rostral to the lesion. In fact, the undulatory movements were most prevalent when the shark was at rest on the bottom and were absent in normal attempts to swim. The two theories need not be mutually exclusive, however. Lissmann's studies showed that dorsal root input is mandatory for the maintenance of undulatory movements. Thus it appears from the present data that the spinal cord of the shark has an inherent undulatory discharge pattern modulated by local sensory input and input from brain centers. If the major effect of the brain on this undulatory discharge pattern is inhibitory, transecting the spinal cord will release the caudal section of spinal cord from these inhibitory influences, thus allowing the inherent discharge pattern to be exhibited in the form of undulatory movements caudal to the lesion site. If the synaptic sites left vacant by spinal cord transection are replaced with excitatory synapses from dorsal root fibers or indigenous spinal tracts by way of sprouting, an increase in the discharge pattern should occur with a resultant increase in the strength of undulatory movements. Undulatory movements in the present experiment began

immediately upon recovery from anesthesia and increased in strength up to 60 days postoperative after which they leveled off. If the postoperative increase in the strength of undulatory movements is compared to the postoperative increase in synaptic complement on motor horn cells caudal to the lesion and the resultant data plotted on a graph (Fig. 8A, 8B), the curves of the two postoperative phenomena are highly correlated. Comparison between boutons on cell bodies and undulatory movements has a correlation coefficient of $r = .930$ ($P < .01$), and comparison between boutons on motor horn cells primary dendrites and undulatory movements results in a correlation coefficient of $r = .91$ ($P < .01$).

It is therefore highly probable from these data that the synaptic return on motor horn cells by way of local sprouting is responsible for the increase in the strength of undulatory activity caudal to the site of lesion. To further support this hypothesis, retransection at 90 days had a minimal effect on both synaptic complement and undulatory movements (Fig. 8A, 8B).

Figure 8 - A. Comparison between synapse count on motor horn cell bodies caudal to the lesion and undulatory strength ($r=.93$, $\underline{p}<.01$).

B. Comparison between synapse count on motor horn cell dendrites caudal to the lesion and undulatory strength ($r=.91$, $\underline{p}<.01$).



CONCLUSION

Regeneration in the shark spinal cord following spinal cord transection appears to lie somewhere between the abortive regeneration usually seen in mammals and the vigorous regeneration and return of function typical of teleosts. Although anatomical regeneration of nerve fibers across the site of lesion does occur, the functional ramifications are negligible and for all practical purposes the shark remains paralyzed caudal to the lesion when attempting to swim. The poor anatomical regeneration and lack of functional return in sharks is surprising. A general rule of thumb which is well documented is that the lower on the phylogenetic scale, the more vigorous and complete is the central nervous system regenerative process. The reasoning for this is that more primitive animals reportedly possess more undifferentiated, pleuri-potential cells capable of differentiation into neural elements. The elasmobranchs occupy the third rung up on the vertebrate phylogenetic ladder immediately below the teleosts, yet show

a postoperative recovery following spinal cord transection more akin to mammals than fish.

Perhaps the most significant and interesting result of this experiment is the strong indication of a functional correlation between the return of synapses on motor horn cells caudal to the lesion and the increase in undulatory strength during the postoperative period. Although functional correlates have been suggested for the phenomenon of sprouting in the mammalian central nervous system (McCouch et al., 1955; Schneider, 1970) the evidence presented in this experiment is perhaps the most conclusive to date of a functional correlate to sprouting in the vertebrate central nervous system.

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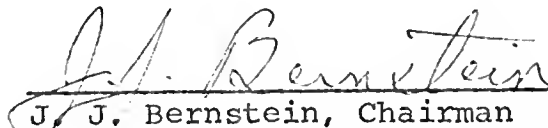
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BIOGRAPHICAL SKETCH


John Bruce Gelderd was born during a German bombing raid in Wasquehal, France on September 21, 1939. He attended grade school in Broadview, Illinois, and Clearwater, Florida, and graduated from Clearwater High School in 1957. After a 4½ year stint in the U. S. Air Force, he attended St. Petersburg Jr. College from September, 1962, to May, 1964. He entered the University of Florida in September, 1964 and received a bachelor's degree in zoology in 1967. He began his graduate studies toward the Doctor of Philosophy degree at the University of Florida in September, 1968.

He has accepted a faculty position in the Department of Anatomy at Louisiana State University in New Orleans, Louisiana.

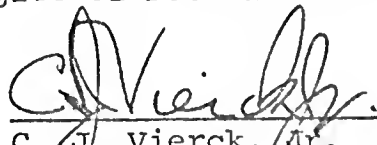
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J. J. Bernstein, Chairman
Associate Professor of Neuroscience

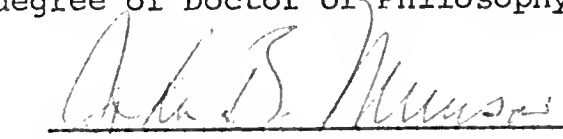
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F. A. King, Chairman of
Department of Neuroscience

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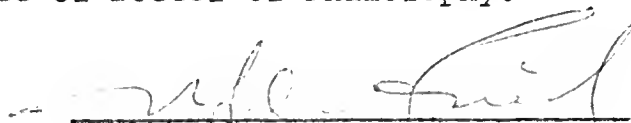

C. J. Vierck, Jr.
Associate Professor of Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


John B. Munson
Assistant Professor, Neuroscience

This dissertation was submitted to the Department of Neuroscience in the College of Medicine and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1972



Dean, College of Medicine

Dean, Graduate School

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